

# THE GAMETOPHYTES OF TAXUS CANADENSIS MARSH

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 234

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(WITH PLATES XI-XIV)

## Introduction

While the European *Taxus baccata* L. has been studied by a number of workers and its morphology is quite well known, up to the present time no accounts have been published dealing in any connected way with the morphology of the American form, *Taxus canadensis* Marsh., records having been made of only a few scattered observations. Although by some, including PILGER (7) in ENGLER'S *Das Pflanzenreich*, *T. canadensis* is considered as merely a subspecies of *T. baccata*, it has seemed worth while to investigate its morphology and compare it with other forms, and especially with the results obtained in the study of *T. baccata*, most of the work in which was done a number of years ago. The present paper gives an account of the male and female gametophytes in connection with other closely related features. Papers dealing with other phases of the morphology will follow.

## Material

The material used in this study was collected during the autumn of 1913 and the years 1914 and 1915 at Huntingdon, Pennsylvania, Sawyer, Michigan, and Mount Carroll, Illinois. Chrome-acetic acid was the chief killing agent used, although some material was killed in formalin-alcohol, but on the whole with results less satisfactory than with the chrome-acetic acid. Heidenhain's iron-alum-hematoxylin, safranin with gentian violet, safranin with light green, and sometimes light green as a contrast stain with the iron-alum-hematoxylin were the stains used. The first of these gave the most satisfactory results in most cases, and for most structures.

### Historical

*Taxus baccata* has long been a favorite form for study, the ovule and the seed first attracting attention, and the early work, therefore, relating almost exclusively to these features. The first study of any importance relating to the gametophytes was that of HOFMEISTER (1), who reported some of the more obvious features of both the male and female gametophytes. The history of the male gametophyte has become further known through the work of BELAJEFF (3), STRASBURGER (4), and JÄGER (6). Miss ROBERTSON (18) made some observations, but, in her own words, her "results on the whole simply confirmed previous work." COKER (9) studied the microspores in *T. baccata* and several of its varieties. STRASBURGER'S early work had to do more especially with the ovulate shoot and the ovule, but in 1879 (2) he described the origin of the megaspore mother cells from the hypodermal layer of the nucellus and gave a few observations on the development of the female gametophyte. The best accounts of the female gametophyte are by JÄGER (6), who gave a rather complete description from the megaspore to the mature endosperm, and by STRASBURGER (12), who gave attention particularly to the early stages, especially to the formation of the megaspores and the free nuclear situation. COKER (9) also studied the megaspores.

In *T. canadensis* itself very little work has been reported. HOFMEISTER (1) has a note concerning the proembryo; CHAMBERLAIN (5) studied the condition of the microsporangium at the beginning of October; COKER (9) reports "more than one embryo sac . . . not uncommon in *Taxus canadensis*"; and THOMSON (15) has studied the condition of the megaspore membrane.

### Male gametophyte

MICROSPORES.—CHAMBERLAIN (5) in material of *T. canadensis* Marsh. collected in the Chicago region, October 1, 1897, found the microsporangium in the spore mother cell stage, and this has been reported (20) as the winter condition. Material collected by the writer, October 10, 1913, shows microspore formation, and material collected at later dates from several localities and in different years in all cases shows the microspores already formed. The

sporangium develops during the summer, and by the time mentioned the microspores are forming (figs. 2-12). In a single strobilus all stages from spore mother cells to completed tetrads may be found. Different stages are also found in a single sporangium, although here the range is not so great as in case of the entire strobilus.

Reduction in the chromosome number and the formation of microspores take place as the result of the usual two divisions of the mother cell. The first division (figs. 2-5) results in two hemispherical cells, and this is followed by the second division (figs. 6, 7), usually in the same plane, resulting in the bilateral tetrad of microspores, although the two planes of division may sometimes be at right angles to one another. Sometimes the second divisions are not simultaneous (fig. 8). Further divisions may also occur, resulting in the formation of more than 4 microspores from a single mother cell, as many as 6 having been found (figs. 9-12). The microspores soon separate from one another and become surrounded by moderately heavy spore coats (fig. 13), the tapetum remaining quite prominent and showing little signs of disintegration even at this time. The microspore stage is the winter condition of the microsporangium, the only noticeable change in the microspores between this and pollination being an increase in size (figs. 13, 14). This growth takes place in the early spring, during the period in which the strobilus is emerging from the winter scales and attaining its mature size (about the middle of April).

*Torreya californica* in England (10) and *T. taxifolia* in Florida (14) pass the winter in the mother cell condition. STRASBURGER (12) reports that in *Taxus baccata* the "pollen mother cells" divided, in 1904, during unusually warm weather in February. So far as reported, *T. canadensis* is the only one of the Taxineae passing the winter in the microspore stage, the microspore mother cell or earlier being the more usual winter condition of our north temperate gymnosperms.

The pollen grain is uninucleate (fig. 14) when shed and shows no indications of prothallial cells, this agreeing with *T. baccata* as reported by several workers (BELAJEFF 3, JÄGER 6, and COKER 9). Among the other Taxineae, *Torreya* (14) and *Cephalotaxus* (4, 16,

17) are binucleate when shed, the microspore having divided into the tube and generative cells while in the microsporangium. The absence of prothallial cells is a feature common to the Taxineae, Taxodineae, and Cupressineae.

POLLINATION.—In the spring of 1914, in the vicinity of Huntingdon, Pennsylvania, pollination was first noted April 23. No strobili were found shedding pollen on April 20, but 3 days later pollen was being shed abundantly, especially in the more exposed situations, and most of the ovules collected at that time contain pollen grains. JÄGER (6), at Zurich, reports the beginning or middle of March as the time for the pollination of *T. baccata*.

The pollen grains are wind scattered and are formed in great abundance, while the frequent occurrence of both staminate and ovulate strobili on the same shoot increases the chances for pollination. In the material which I have examined there were very few ovules which had not been pollinated, and sometimes quite abundantly, as is evidenced by the frequency of several pollen tubes in an ovule. The pollen is caught by the small pollination droplet which protrudes from the micropyle of the ovule and is drawn into the micropyle by the concentration of the droplet.

In the ovule the pollen grains are found on the upper end of the nucellus. While no definite pollen chamber is formed, the outermost cells of the nucellus begin to disintegrate about the time of pollination, resulting in a ragged edge to the nucellus on which the pollen grains become lodged.

POLLEN TUBE.—The pollen grain, lying on the nucellus, soon begins to elongate, the exine is ruptured, and the intine grows out to form the tube. The young tube soon penetrates the nucellus, and, once started, invades the tissue very rapidly. Within a week or 10 days some of the tubes have reached the female gametophyte, while others may not have penetrated so far, there being considerable variation in this respect even in a single ovule, although the rapid growth is the more general situation (figs. 16–20, 22).

The general course of the pollen tube is toward the region of the female gametophyte, which occupies the center of the nucellus. However, it is not uncommon for tubes to penetrate the nucellus

nearer its margins, in some cases reaching close to the edge of the nucellus (fig. 27). During the elongation of the tube there is also considerable increase in diameter, such that by the time the tube has reached the female gametophyte region its diameter is several times greater than at first (fig. 22). A striking enlargement now takes place. If the female gametophyte still consists of free nuclei, as it frequently does at this time, it may be so crowded upon by the enlarging tube as to be pushed to one side; or the megaspore membrane may remain quite firm, retaining its shape, the tube then being forced to conform its shape to that of the resisting megaspore membrane (fig. 28). A number of cases of branching tubes were found, the nuclear contents in such cases being near the point of branching, while the branches generally have dense cytoplasmic contents, indicating their haustorial activity. The enlarging tube usually spreads itself over the micropylar end of the female gametophyte; it may go off to one side and penetrate far into the nucellus; or it may grow along the side of the endosperm, passing by the archegonia in the micropylar end of the endosperm. If two female gametophytes are formed in an axial row, the pollen tube usually enlarges between them, while in some cases the pollen tube may entirely pass the developing female gametophyte and enlarge below it. It seems that in such cases the female gametophyte is developing from an upper megaspore (fig. 31).

In case the nucellus is invaded by a number of tubes they produce a rather interesting situation. The female gametophyte is crowded upon from several sides and is frequently pressed quite out of its normal shape. A case was found of an ovule which has no fewer than 22 pollen tubes, as indicated by their nuclear contents (fig. 32). The pollen tubes have completely invested the endosperm, considerably distorting it, and possibly preventing its normal development, although young archegonia are recognizable in it. In another case at least 9 tubes had enlarged about the endosperm, practically surrounding it, but in this case 3 of the archegonia had been fertilized and proembryos formed within them.

Apparently the tubes do not fuse even when in contact with one another, although when there are a number of tubes in an ovule

their enlargement and crowding upon one another produces such a complex that it is practically impossible to count the tubes except by their nuclear contents. The tubes which are on the periphery of the complex may be so crowded by the more interior ones as to remain quite narrow and their contained body cells may even be flattened by the pressure.

The cytoplasmic contents of the tube become quite vacuolated early in the growth of the tube and in early stages contain a large number of starch grains, although these apparently disappear in the later stages. The cytoplasm is always more abundant near the growing end of the tube, but when the tube enlarges at the forward end the bulk of the cytoplasm remains more or less closely connected with the nuclei of the male gametophyte. Frequently in late stages the cytoplasm may contain accumulated masses, irregular in shape and densely staining, and these may even be discharged into the egg cytoplasm at the time of fertilization.

**DEVELOPMENT OF MALE GAMETOPHYTE.**—Soon after pollination the microspore begins to elongate, and the first division into tube and generative cells takes place within 10–12 days after pollination (fig. 16). This division results in two unequal cells (fig. 17), the smaller generative cell being held in the base of the grain by a plasma membrane, while the larger tube cell forms the elongating tube. The division of nuclear material is no doubt equal, but the tube cell nucleus soon becomes larger than the nucleus of the generative cell, the former becoming ellipsoid and retaining this shape throughout the remainder of its history. The cytoplasm of the generative cell is denser than that of the tube cell.

As the tube elongates, the tube cell nucleus passes into the growing end, migrating rapidly behind the tip of the tube (figs. 19, 20). Even before the generative cell divides, the tube cell nucleus has usually gone some distance into the tube. The generative cell enlarges, pushes out its limiting membrane, and then divides. No case was found showing the second division, but that the two nuclei found together in the basal part of the tube (fig. 20) arose from the division of the generative cell can hardly be doubted. The basal one, the stalk cell, has a more vacuolated cytoplasm than that of the anterior body cell.

The body cell soon migrates into the tube and is followed by the stalk cell nucleus, the two usually being in contact and apparently having passed into the tube together. During the growth of the tube through the nucellus, up to the time when the pollen tube reaches the female gametophyte, the 3 nuclei are in an axial row, while later the stalk nucleus migrates around the body cell and takes a position besides the tube nucleus. Of these two nuclei in the tube, the tube nucleus is generally to be recognized by its being slightly larger than the stalk nucleus. The body cell begins enlarging at once after entering the tube. As it passes down the tube it is slightly ellipsoid, but on reaching the end of the tube, as the latter is enlarging about the endosperm, it becomes rounded as it increases in size. The cytoplasm remains dense and the nucleus becomes large. During the early history of the body cell its cytoplasm is uniformly distributed through the cell, but as it reaches its mature size the cytoplasm becomes less dense along the periphery and here shows delicate radial strands (fig. 23). The nucleus, which earlier occupied a central position, takes a more peripheral one (usually on the side opposite to that on which the stalk and tube nuclei lie), while the cytoplasm becomes considerably vacuolated along the margin of the cell, the radial strands disappearing with the increased vacuolization.

The division of the body cell into the two male cells takes place shortly before fertilization. Several mitotic figures of this division were found. The nucleus of the body cell being at one side of the cell, there is an unequal division of the cytoplasmic material. A broad spindle is formed and the cell plate laid down on it is lenticular in outline, resulting in the formation of the two unequal male cells, a small lens-shaped cell and a larger more rounded one. The nucleus of the larger cell takes a central position and enlarges, while with the growth of the nucleus of the smaller cell the latter soon has the appearance of a naked nucleus invested only by the nuclear membrane, the two cells remaining in contact with one another and being held together by the plasma membrane of the parent body cell (figs. 23-26). What is probably a similar situation in *Taxus baccata* has given rise to differences in the accounts as given by different workers. BELAJEFF (3) could not find a plasma

investment of the smaller nucleus. He speaks of "male nuclei" and not of male cells. His figure shows two nuclei in a common cytoplasmic mass. STRASBURGER (4) and JÄGER (6) both recognized two "cells." Miss ROBERTSON (18) figures the division of the body cell, but no cell plate is shown on the spindle, even in the late telophase; while her figure of the completed division lacks clearness on this point, owing to a possible inaccuracy in drawing or in technique. In her discussion she speaks of a "functional male nucleus" and "inequality of the sperm nuclei."

By the time the two male cells are formed, the vacuolization along the margin of the larger cell has become quite pronounced (fig. 26), and this continues until finally the cytoplasm has practically all withdrawn from the plasma membrane and collected about the nucleus. In this condition the male gametophyte has reached its maturity and fertilization may now take place. Should male cells fail to function in fertilizing an egg, as is frequently the case where there are a number of tubes in an ovule, they remain in this condition for a time and then disintegrate. Male cells have been found in ovules in which the proembryo was considerably advanced.

Two unequal male cells are reported for *Torreya taxifolia* (14) and *Cephalotaxus Fortunei* (16), and the division of the body cell into male nuclei in *C. drupacea* (17). Miss ROBERTSON concludes that there are formed "a functional male nucleus" and a "smaller male nucleus" in *T. californica* (18). In an earlier account (11) she had stated that the body cell divided into two nuclei of equal size. There is evident among the Taxineae a tendency toward the elimination of male cells in the formation of the so-called "male nuclei" only. The inequality of these nuclei or cells is another advance. A mere cutting off of the smaller nucleus from the body cell would be another step; while the final one, not known to be reached by any of the gymnosperms, would be a body cell functioning as a male cell. In *Taxus* the male gametophyte at its maturity consists of the stalk and tube nuclei and the two male cells. This is interesting in comparison with the angiosperm situation, in which the generative cell in its division produces the two male cells directly, thus eliminating one more



division between the microspore and the cells functioning in fertilization.

### Female gametophyte

MEGASPORES.—STRASBURGER (2) long ago pointed out that in *Taxus baccata* the megaspore mother cells are the end cells of a series arising from the hypodermal layer of the nucellus, and that they are clearly distinguished from the surrounding cells by their larger size and larger nuclei. The same situation seems to hold for *T. canadensis*. STRASBURGER (2) claimed several megaspore mother cells; JÄGER (6) agrees with him, but COKER (9) in his study of *T. baccata* states that there is no evidence that more than one megaspore is ever formed. He says "the mother cell is hard to distinguish. At the time of its first division it is long and narrow, resembling very closely the cells adjoining." As to the number of mother cells, STRASBURGER was probably right. In my preparations I have found no difficulty in recognizing the megaspore mother cells, nor does there seem to be any doubt that there may be a number of them in an ovule. Only one, or occasionally two, may function, but other megaspore mother cells may be present, by all the other tests of a mother cell. The mother cells are distinguished from the other cells of the nucellus, not only by their size and the size of their nuclei, but by their different staining reaction. The group of mother cells may be recognized in the autumn or winter (fig. 33). The occurrence of two linear tetrads of megaspores (fig. 38) is sufficient evidence that there have been at least two megaspore mother cells in the case figured.

STRASBURGER (12) and COKER (9) have given rather complete descriptions of megaspore formation in *Taxus baccata*, and the process is essentially the same in *T. canadensis*, so far as my preparations show (figs. 34-38). In *T. baccata* the second division is said to be simultaneous in both daughter cells. This is probably the case in *T. canadensis* in most instances, although one case was found in which the division of the upper cell was completed before that of the lower (fig. 36).

Starch is present in considerable abundance in the megaspore mother cells as well as in the neighboring cells, and it is also found in the megaspores and in the developing female gametophyte.

STRASBURGER (12) states that the starch soon disappears from the megaspore mother cells of *T. baccata*, but COKER (9) in his figures shows starch grains in the megaspores as well as in the megaspore mother cells. The starch is not confined to the megaspore region, but is found abundantly in the adjoining cells, and some of it occurs throughout a considerable portion of the nucellus in *T. canadensis*.

The method of formation of a tapetum about the megaspore mother cells, as pointed out by STRASBURGER (12) in *T. baccata*, also holds for *T. canadensis*. These cells are formed more or less obliquely to the long axis of the megaspore cells (fig. 34), are rich in content, and stain differently from the megaspore mother cells. THOMSON (15) speaks of the tapetum derived from the nucellus as a "secondary tapetum," in distinction from forms in which the tapetum is derived from the sporogenous tissue, in which case it is called a "primary tapetum." GOEBEL pointed out long ago, however, that the significance of the "tapetum" is physiological, and not morphological, and that it may have a variety of origins. The megaspore mother cell seems to be the usual winter condition. While STRASBURGER (12) says the megaspore mother cells of *T. baccata* are completed in October and that further development takes place the next spring, this does not always hold for *T. canadensis*, as I have found young female gametophytes of several free nuclei in some of the material collected in November, 1913. Most of the ovules taken at this time showed the mother cell condition, but even in some of the ovules collected as late as December it may be doubted whether the megaspore mother cells had become fully matured. Evidently, therefore, the time for the maturity of the megaspore mother cells and the formation of megaspores may vary, yet the general statement may be made that the mother cell is the usual winter condition and that megaspore formation generally takes place in the spring with the renewal of the growing season.

Usually the innermost one of the linear tetrad of megaspores is the functional one, the 3 outermost then disorganizing and disappearing sooner or later (fig. 39). Any of the megaspores may function, however, as 1, 2, 3, or even all 4 of the megaspores may grow after their formation and more than one may divide in

the formation of young female gametophytes (figs. 39-44). The further development of several megaspores will be described later. Some interesting cases were found, such as those in which one of the upper megaspores had evidently functioned, while the lower ones had failed to develop, although still recognizable (figs. 30, 31). In both cases figured the pollen tubes have pushed past the gametophytes and penetrated to the central region of the nucellus.

THOMSON (15) in his investigation covering the megaspore membrane situation in the gymnosperms reports that in *Taxus canadensis* the megaspore membrane, while recognizable in the early free nuclear stages of the gametophyte, is practically unrecognizable in later stages. This agrees with my observations that the membrane is quite firm about the young female gametophyte, but seemingly fails to develop with the endosperm and is soon lost sight of. THOMSON associates this with the absence of the "primary tapetum" and regards it as a specialized advanced character, indicating that the Taxineae are "recent" as compared with some other forms.

#### DEVELOPMENT OF FEMALE GAMETOPHYTE

*Free nuclear stage.*—The first division of the functional megaspores takes place soon after their formation, and other divisions, which are always simultaneous, follow in rapid succession (figs. 39, 45, 46). The embryo sac enlarges as the number of nuclei increases. At first the nuclei are scattered in the embryo sac, but as this increases in size it becomes vacuolated in the center, the cytoplasm with the nuclei then taking a peripheral position, this condition being attained when the 8 or 16-nucleate stage is reached (figs. 47, 48). As reported by JÄGER (6) for *Taxus baccata*, about 8 successive divisions occur, resulting in approximately 256 free nuclei before wall formation takes place. The vacuole in the embryo sac has kept pace with the growth of the sac and the nuclei occur in the narrow cytoplasmic layer along the periphery, the cytoplasm not being uniformly distributed, however, but showing distinct radiations connecting the nuclei when seen in a tangential view (fig. 49).

In the enlargement of the embryo sac, if a lower megaspore is functioning, the growth seems to be downward; if an upper megaspore is functioning, the growth is upward; if two gametophytes

are developing, one grows upward, the other downward (fig. 51). In the early stages the embryo sac is usually pear-shaped, the narrow portion marking the original position of the megaspores, while the expanded portion shows the region of growth (figs. 47, 51). The growth of the embryo sac and the enlargement of the endosperm after walls have been formed crowd upon the adjoining cells of the nucellus in such a way as to distort and flatten them, while no doubt some of the nucellar tissue is also digested by the growing gametophyte.

*Wall formation and growth of endosperm.*—The first formation of walls between the nuclei results in a single layer of cells surrounding the central cavity (fig. 50). The cells at this stage are rich in starch, the starch grains having also been present during the free nuclear stages. Centripetal growth of these cells then begins by the radial lengthening of their walls, the walls reaching the center and forming a completely closed tissue before further cell division takes place. Several cases were found showing this feature in various stages, but in no case had periclinal walls formed before the tissue was closed (fig. 51). Cells which in a single section appear to be internal are merely the inner ends of cells abutting the margin in other sections. At the very narrow upper end the cells are very closely crowded together.

JÄGER'S account (6) of the formation of the endosperm in *Taxus baccata* differs in a few details from this, in that he states that the cavity is filled with tissue by the inward growth of a series of cells formed by periclinal walls. His technique was such, however, that he could easily have been mistaken in his interpretation of the situation. The filling of the central cavity by growth and periclinal divisions seems to be the rule among gymnosperms, and is probably to be correlated with the size of the cavity at the time wall formation begins. The smallness of the cavity in *Taxus* may account for the method of tissue formation found here.

Following the complete filling of the central cavity with cell tissue, periclinal walls come in, giving rise to several layers of cells between the margin and the center. Anticlinal divisions also take place soon and the growth of the endosperm in all directions continues. Archegonia soon appear in the micropylar end of the tissue.

Growth of the endosperm goes on rapidly, the greater meristematic activity being in the central portion, especially in the basal region. In the early history of the endosperm the cells are uninucleate, but as the embryo develops they become multinucleate, while in the central portion of the endosperm, below the growing tip of the embryo, the cells become elongated, forming a conducting tract for the food from the basal region of the ovule to the growing embryo. This elongation ceases to show after the embryo reaches maturity. In the mature endosperm, with the exception of the extreme micropylar portion, an abundance of food material is stored, this being the food supply of the seedling in the early stages of its germination.

A comparison of the size of the endosperm at different times in its development may be of interest. In fig. 52 there are shown 3 outline drawings, to the same scale, showing the comparative size of the endosperm at the time of wall formation (as in fig. 51), at the time of fertilization, and at the maturity of the seed. It is readily seen that the greatest growth of the endosperm takes place after fertilization.

*Archegonia.*—The archegonium initial arises from the outermost layer of cells and is recognizable very shortly after periclinal walls come in. The initials appear a short distance behind the "point" of the endosperm, but always occur in the micropylar end if the gametophyte is one which has developed from an inner megaspore; in case of a gametophyte from an outer megaspore the archegonia will be on the side of the gametophyte toward the center of the ovule. The initials can be recognized by their slightly larger size and by the size of their nuclei (fig. 53). The surrounding cells form the archegonial jacket. The initial divides into the primary neck cell and the central cell (fig. 54). The primary neck cell divides into several neck cells, all in the tangential plane, serving merely as a covering for the central cell, a "neck" hardly being recognizable. These neck cells become flattened, and by the time of the maturity of the archegonia are mere plates, usually with degenerate nuclei and but little cytoplasm.

The central cell enlarges to the mature size, and since in the large number of archegonia examined there were no indications of a

ventral canal cell or nucleus, it may be regarded that this central cell is the functional egg. This agrees with *Torreya taxifolia* (14). In *Cephalotaxus Fortunei* (15) and *C. drupacea* (16) a ventral nucleus is formed, in the latter disorganizing before fertilization. This marks the final elimination of the row of canal cells, an elimination which has been such a persistent and gradual process from bryophytes through pteridophytes and gymnosperms.

In the earlier stages of the archegonium the central cell nucleus is near the upper end of the cell, but as the archegonium matures it takes a more central position (fig. 55), the cytoplasm being somewhat vacuolated and supplied with an abundance of food material, some of which stains quite darkly with the staining agents used.

The archegonial jacket is recognizable from the initial to the mature archegonium, but is not strikingly conspicuous as in some other forms, and less so in the mature condition than earlier. Usually there is a jacket about each archegonium, with several layers of cells between the archegonia, but it is not a rare thing to see two archegonia with only a single layer of jacket cells between them, and several cases were found in which two archegonia were surrounded by a common jacket, this latter condition being an approach to the archegonium complex found in some of the other groups of gymnosperms. Several archegonia are usually present in a gametophyte, 4-8 being the average number.

**SUPERNUMERARY GAMETOPHYTES.**—Mention has been made of the fact that more than one megaspore may function. HOFMEISTER (1) long ago pointed out the presence of more than one embryo sac in *Taxus baccata*, and JÄGER (6), STRASBURGER (12), and Miss ROBERTSON (18) have found the same situation. STRASBURGER states that usually one embryo sac develops; if more than one starts, one is usually suppressed; but several times he observed two equally strong embryo sacs with more or less free peripheral nuclei, the two embryo sacs usually lying beside one another, and one case of one above the other. COKER (9) and THOMSON (15) found more than one embryo sac in *T. canadensis*, the former stating that one of the two gametophytes is smaller than the other, and while both may bear archegonia, the archegonia in the upper gametophyte face the pollen tube which has pushed in between the

two gametophytes, only the archegonia of the lower gametophyte, however, being fertilized.

In my material I find two gametophytes quite common, usually in an axial row, although sometimes lying side by side (fig. 57). One is usually larger than the other, the upper generally being the smaller of the two, and both may produce archegonia, as pointed out by COKER. Usually when 2 gametophytes develop in an axial row the pollen tubes push in between them, and then the archegonia are directed toward the tubes; but this is not always the case, as sometimes there are 2 apparently equally vigorous gametophytes, one above the other, and both with good archegonia in their micropylar ends. The pollen tube has spread out above the upper gametophyte, while the lower one is not in contact with the tube at any point in the case shown in fig. 58.

Several instances of more than 2 gametophytes in an ovule were found. One case (fig. 59) shows 3 gametophytes with tissue, the pollen tube lying between the two uppermost. No archegonia were present in the upper one, but both of the lower ones have good archegonia, one of which has been fertilized and contains a proembryo. Other cases show 4 gametophytes, in one of which (fig. 60) 2 of the gametophytes have formed tissue, while the other 2 are in free nuclear condition, archegonia being present only in the lowest, which also contains a proembryo, the pollen tube in this case having pushed its way between the 4 gametophytes. In another case (fig. 61) the ovule contains 3 gametophytes with tissue and archegonia, while the fourth one consists of only a few free nuclei and, although lying between two vigorous gametophytes with tissue, retains its shape, evidently owing to the firmness of the megaspore membrane. One ovule was found containing 5 gametophytes, 3 with free nuclei and 2 with tissue (fig. 62), only the lowest of the 5 having developed an archegonium. In this case more than one megaspore mother cell must have functioned. With two tetrads of megaspores (fig. 38) a situation such as this, and even with more gametophytes, is not impossible.

The mechanics by which the developing gametophytes become separated by the enlarging tube might be an interesting problem,

to which it may be suggested that there is an elongation of the nucellus during the growth of the gametophytes, together with a digestion of that portion of the nucellus immediately adjacent to them. The megaspores were evidently in contact when formed, but by the enlargement of the pollen tube between the young gametophytes they become widely separated as the tube develops.

### Fertilization

While the fertilized egg represents a new phase in the life history and the account of it might be more properly included with that of the embryogeny, it may not be out of place to give a brief account of it in this connection. Ovules containing proembryos were found in material collected May 21. Preparations showing fertilization were found from this time on to as late as the middle of June, showing that the time for fertilization is not constant and may have considerable range.

At the time of fertilization the neck of the archegonium becomes ruptured and the nuclear contents and part of the cytoplasmic contents of the tube are discharged into the egg. The egg nucleus has migrated to the basal portion of the egg; the male nucleus with its investing cytoplasm comes in contact with the egg nucleus; the cytoplasm of the male nucleus invests the two nuclei lying in contact and forms a dense sheath about them (fig. 56). With the fusion of the two nuclei the act of fertilization is complete. The behavior of the chromatin in fertilization could not be determined from my material. The cytoplasmic sheath about the two nuclei also occurs in *Torreya californica* (11), *T. taxifolia* (14), and *Cephalotaxus Fortunei* (16) among the Taxaceae, as well as in several other cases reported, namely, by COKER (8) in *Taxodium*; by LAND (13) in *Ephedra*; and by NICHOLS (19) in *Juniperus communis* var. *depressa*.

The smaller male cell and the stalk and tube nuclei, together with the densely staining mass of the tube cytoplasm, when present, usually remain in the upper portion of the egg, where they sooner or later disorganize, although they may persist for some time and be recognizable at somewhat late stages of the proembryo or even after the suspensors have begun to elongate.



### Time relations

The time periods involved in the reproductive process are always of interest in the gymnosperms, as use is made of this feature in determining the primitive or modern character of a group, those having short periods being regarded as the more advanced in this respect. In my material the time from microspore formation to pollination was about 6.5 months; from pollination to fertilization may be as short as one month, although fertilization generally occurs after a longer interval, the time between pollination and fertilization having a considerable range, with an accompanying range in the time of the maturity of the seed. I have collected mature seeds from the first week of July until late in September in central Pennsylvania. BELAJEFF (3) shows a pollen tube of *Taxus baccata* with two nuclei collected April 10 and a figure of fertilization dated May 26. JÄGER (6), at Zurich, reports pollination at the beginning or middle of March and fertilization at the end of May or beginning of June. Miss ROBERTSON (18) reports the time for fertilization at Kew to be about the middle of June. STRASBURGER (4) speaks of fertilization taking place the first half of July and the embryo complete by the end of August. None of these accounts gives so short a time period as I have found for *T. canadensis*.

### Summary

Microspore formation takes place in the autumn. There are no indications of prothallial cells. The pollen grain is uninucleate when shed.

The pollen tube penetrates the nucellus very rapidly and enlarges excessively about the female gametophyte.

Three divisions take place in the development of the male gametophyte. The body cell divides into two unequal male cells, the larger of which functions in fertilization.

Several megaspore mother cells are formed, of which only one usually functions, although two of them may form megaspores. The megaspore mother cell is the usual winter condition, but megaspores may be formed and the female gametophyte may consist of several free nuclei before winter.

Following the free nuclear stage of the female gametophyte, radical walls come in, closing the cavity before the appearance of periclinal walls.

The archegonia appear early in the endosperm. The central cell is the functional egg, no ventral canal cell or ventral nucleus being formed.

More than one female gametophyte in an ovule is common; as many as 5 were observed.

In fertilization the nuclear contents of the pollen tube are discharged into the egg. A cytoplasmic sheath is formed about the two fusing nuclei.

From pollination to fertilization may be as short as one month. Mature seeds have been collected 6 weeks later.

In general the gametophyte history agrees with that reported for *T. baccata*.

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#### EXPLANATION OF PLATES XI-XIV

All drawings were made with a camera lucida and the figures illustrating a series of processes are drawn to the same scale in order to show comparative sizes. The original drawings are here reduced one-half. The magnification of the figures is approximately as follows: figs. 1-21, 23-26,  $\times 570$ ; fig. 22,  $\times 92$ ; figs. 27-32, 57-62,  $\times 57$ ; figs. 33-51, 53-56,  $\times 268$ .

#### PLATE XI

FIG. 1.—Group of megaspore mother cells.

FIG. 2.—Synapsis preceding reduction.

FIGS. 3-7.—Stages in reduction division.

FIG. 8.—Two microspores are formed; second division in formation of other two not yet completed.

FIG. 9.—Complete tetrad of 4 microspores.

FIGS. 10-12.—Tetrads of more than 4 microspores, fig. 12 showing 6 microspores.

FIG. 13.—Portion of a microsporangium, showing wall, tapetum, and several microspores in winter condition.

FIG. 14.—Mature microspore at time of shedding.

FIG. 15.—Pollen grain after pollination has taken place, beginning to elongate.

FIG. 16.—First division of microspore.

FIG. 17.—Smaller generative cell and larger tube cell resulting from first division of microspore.

FIG. 18.—Stage slightly more advanced than preceding figure; tube nucleus passing into tube and cytoplasm becoming vacuolated.

FIG. 19.—Somewhat older tube, showing generative cell in forward end of tube enlarging before division, and large number of starch grains in cytoplasm of tube.

FIG. 20.—Generative cell has divided into basal stalk cell and anterior body cell, which already shows denser cytoplasmic contents than stalk cell.

FIG. 21.—Passage of stalk and body cells into tube.

FIG. 22.—Portion of an ovule (reconstructed from several sections) showing female gametophyte in nucellar tissue and several pollen tubes which have penetrated nucellus to female gametophyte.

FIG. 23.—Body cell at time of maturity, with laterally placed nucleus and delicate cytoplasmic radiations along periphery, together with stalk cell nucleus and tube nucleus.

FIG. 24.—Division of body cell.

FIG. 25.—Late stage in division of body cell; lenticular cell plate is shown, as well as vacuolization along margin of cell.

FIG. 26.—Mature male gametophyte, consisting of two unequal male cells, stalk cell nucleus, and tube nucleus.

#### PLATE XII

FIG. 27.—Portion of an ovule in which a branching pollen tube has passed to one side of nucellus.

FIG. 28.—Portion of an ovule showing more usual relation of pollen tube to female gametophyte.

FIG. 29.—Portion of an ovule in which pollen tube has passed to one side of female gametophyte, penetrating nucellus nearly to base of female gametophyte.

FIG. 30.—Portion of an ovule in which female gametophyte has developed from an upper megaspore, pollen tube having pushed past gametophyte.

FIG. 31.—Two pollen tubes have pushed past female gametophyte and enlarged below it; non-functioning lower megaspore is recognizable.

FIG. 32.—An ovule which contained no less than 22 pollen tubes; the figure is a reconstruction of several sections and shows the complex about female gametophyte and nuclear contents of several tubes.

FIG. 33.—Group of several megaspore mother cells.

FIG. 34.—Synapsis preceding reduction division of megaspore mother cell; division showing method of formation of tapetum.

FIG. 35.—The 2 cells resulting from first division of one of the two megaspore mother cells.

FIG. 36.—Megaspore tetrad, division of two lowest as yet incomplete.

FIG. 37.—Linear tetrad of megaspores.

FIG. 38.—Two linear tetrads of megaspores.

FIG. 39.—Innermost megaspore dividing, 3 outer ones degenerating.

FIG. 40.—Two-celled female gametophyte from division of inner megaspore; second megaspore has enlarged.

FIG. 41.—Inner megaspore divided, other 3 showing no signs of disintegration.

PLATE XIII

FIG. 42.—Four megaspores have enlarged considerably and nucleus of one has divided.

FIG. 43.—Second of the 4 megaspores has divided; first has enlarged somewhat, the two innermost retaining their normal size and appearance.

FIG. 44.—Third and fourth megaspores have each divided twice, second megaspore has enlarged, while outer one is beginning to degenerate.

FIG. 45.—Second division of megaspore.

FIG. 46.—Third division of megaspore; note beginning of formation of pear-shaped embryo sac.

FIG. 47.—An 8-nucleate female gametophyte.

FIG. 48.—Group in which first and third megaspores are disintegrating, second has enlarged considerably, while the fourth has produced an 8-nucleate female gametophyte.

FIG. 49.—Tangential view of a few nuclei and connecting cytoplasmic strands in free nuclear condition of female gametophyte.

FIG. 50.—Female gametophyte showing single layer of cells following first wall formation.

FIG. 51.—Two female gametophytes in axial row; upper one in free nuclear condition, lower with tissue just completed, completely closing embryo sac cavity; no periclinal walls have appeared, cells showing in interior being merely portions of cells whose outer ends abut on margin.

FIG. 52.—Female gametophyte at 3 different stages, at time of complete closing of cavity (fig. 51), at time of fertilization, and at maturity of seed, drawn to same scale, to show comparative size at different stages.

PLATE XIV

FIG. 53.—Upper portion of a female gametophyte showing 2 archegonium initials.

FIG. 54.—Two archegonia, showing young central and primary neck cells.

FIG. 55.—Mature archegonium; nucleus has become centrally placed, cytoplasm shows more or less radiations from nucleus and contains considerable food material; nuclei of neck cells degenerating.

FIG. 56.—Fertilization: male nucleus and egg nucleus are in contact at base of egg, surrounded by cytoplasmic sheath; smaller male cell nucleus and tube and stalk cell nuclei can be seen in upper portion of egg cytoplasm; ellipsoid dark bodies are food particles, while irregular dark mass seen in upper portion of egg is an accumulated mass of cytoplasm from pollen tube.

FIG. 57.—Two female gametophytes lying side by side, with innermost portions of 2 pollen tubes beside them.

FIG. 58.—Two gametophytes in axial row, both having developed good archegonia; pollen tube has spread out over micropylar end of outer gametophyte.

FIG. 59.—Three female gametophytes in axial row, with pollen tube between first and second; second contains a proembryo, while inner one also contains a good archegonium.

FIG. 60.—Ovule containing 4 female gametophytes, 2 of which are in free nuclear condition; lower one contains a proembryo; pollen tube has crowded between the 4 gametophytes.

FIG. 61.—Another ovule with 4 female gametophytes, 3 of which contain archegonia; one of gametophytes consists of only a few free nuclei, but has not been pressed out of shape by its neighbors; pollen tube is shown below upper gametophyte.

FIG. 62.—Portion of ovule with 5 female gametophytes, 3 of which are in free nuclear condition, and an archegonium appearing only in lower of two with tissue; enlarging pollen tube has widely separated 3 upper gametophytes from 2 lower ones.